



**UNIVERSITI PUTRA MALAYSIA**

**HEAT SHOCK PROTEIN 70 AND HEAT TOLERANCE IN EARLY-AGE  
FEED RESTRICTED BROILER CHICKENS**

**CHE NORMA MAT TAIB**

**FP 2000 17**

**HEAT SHOCK PROTEIN 70 AND HEAT TOLERANCE IN EARLY-AGE  
FEED RESTRICTED BROILER CHICKENS**

**BY**

**CHE NORMA MAT TAIB**

**Thesis Submitted in Fulfilment of the Requirements for the  
Degree of Master Science in the Faculty of Agriculture  
Universiti Putra Malaysia**

**January 2000**



**In the name of Allah, the Beneficial, the Merciful**

**To**

**AYAH & BONDA**

**"In the creation of the heavens and the earth and in the alteration of the night and the day there are indeed signs for people of understanding".**

**(Al-Qur'an, V:190-191)**

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in  
fulfilment of the requirements for the degree of Master of Science

**HEAT SHOCK PROTEIN 70 RESPONSE AND HEAT TOLERANCE IN  
EARLY-AGE FEED RESTRICTED BROILER CHICKENS**

By

**CHE NORMA MAT TAIB**

**January 2000**

**Chairman: Associate Professor Dr. Zulkifli Idrus, Ph.D.**

**Faculty: Agriculture**

The objectives of this study are to evaluate the effect of various degree of neonatal feed restrictions on heat tolerance later in life, the importance of heat shock protein 70 (HSP 70) in eliciting thermotolerance in broilers and the relationship between heat stress and occurrence of programmed cell death (apoptosis). Broiler chicks that were subjected to 80% feed restriction (F80), 60% feed restriction (F60) and 40% feed restriction (F40) or *ad libitum* feeding from 4 to 6 days of age were exposed to high ambient temperatures ( $38\pm1^{\circ}\text{C}$ ) for 2hr/day from 35 to 42 days of age.

Short term feed restriction during the first week of life caused retardation of growth. Although feed restriction reduced initial growth, birds grew more rapidly than those fed *ad libitum* (AL) during refeeding. One day following the imposition of feed limitation, higher levels of HSP 70 expression in the brain tissues and increased heterophil/lymphocyte (H/L) ratios were noted among F60 and F40 birds.

Birds subjected to fasting early in life (F60) improved HSP 70 expression, growth, survivability, and reduced H/L ratios compared to those fed AL and F80 in response to the heat treatment. The survivability rate and H/L ratios of F40 chicks were similar to those attained by other feeding regimens (AL and F80). Irrespective of feeding regimen, heat stress resulted in an increase in serum glucose level and appearance of programmed cell death (apoptosis) in the thymus glands. These results suggest that neonatal fasting evokes heat tolerance later in life through enhanced expression of HSP 70. Exposing birds to feed restriction of either lower (F80) or higher (F40) severity do not have profound influence on subsequent resistance to heat stress later in life.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia bagi memenuhi syarat untuk Ijazah Master Sains

**GERAKBALAS "HEAT SHOCK PROTEIN 70" DAN KETAHANAN  
TERHADAP TEGASAN HABA PADA SEKATAN MAKANAN DI  
PERINGKAT AWAL HAYAT AYAM PEDAGING**

Oleh

**CHE NORMA MAT TAIB**

Januari 2000

**Penerusi: Prof. Madya Dr. Zulkifli Idrus, Ph.D.**

**Fakulti: Pertanian**

Eksperimen dijalankan ke atas ayam pedaging bagi menilai kesan beberapa tahap sekatan makanan di peringkat awal hayat terhadap ketahanan sistem badan terhadap tegasan haba yang dikenakan di peringkat akhir hayat, untuk memastikan kepentingan "HSP 70" dalam mengawal tegasan haba pada ayam dan juga untuk menilai hubungan kait diantara tekanan haba dengan pembentukan sel mati terancang ("apoptosis"). Anak ayam diletakkan di bawah pengaruh sekatan makanan iaitu sama ada pengambilan makanan tanpa had (AL), 80% sekatan makanan, 60% sekatan makanan dan 40% sekatan makanan. Sekatan makanan ini dilakukan bermula dari umur 4 hingga 6 hari dan kemudiannya bermula dari umur 35

hingga 42 hari, ayam tersebut kemudian didedahkan pula kepada tegasan haba setinggi  $38^{\circ}\pm 1^{\circ}\text{C}$  selama 2 jam setiap hari.

Sekatan makanan yang dibuat untuk jangka masa yang singkat di peringkat awal menyebabkan berlakunya terbantut pertumbuhan berat badan. Meskipun begitu, selepas sekatan tersebut dihentikan, ayam yang mengalami sekatan makanan menunjukkan kadar pertumbuhan berat badan yang lebih baik berbanding dengan ayam yang tidak mengalami sebarang sekatan makanan. Sehari selepas tekanan makanan dijalankan, tahap penghasilan "HSP 70" dalam tisu otak dan nisbah sel heterofil kepada sel limfosit (H/L) dalam darah meningkat bagi ayam yang berada dalam kumpulan F60 dan F40 sahaja. Manakala ayam dari kumpulan F80 masih tidak menunjukkan sebarang perubahan iaitu masih sama dengan ayam kumpulan (AL).

Ayam yang didedahkan kepada 60% sekatan makan di peringkat awal hayat menunjukkan peningkatan penghasilan "HSP 70", kadar pertumbuhan, tahap kehidupan serta mengurangkan nisbah sel H/L jika dibandingkan dengan ayam dari kumpulan lain iaitu (AL dan F80) apabila mereka didedahkan kepada tekanan haba di akhir hayat. Manakala kadar kehidupan dan nisbah sel H/L bagi F40 adalah sama untuk kesemua ayam dari kumpulan lain. Tanpa bersandarkan, kepada tahap sekatan makanan di

peringkat awal hayat, peningkatan suhu badan dari hasil pendedahan kepada suhu panas dapat menaikkan kandungan glukosa dalam darah dan menyebabkan terbentuknya "apoptosis" pada sel kelenjar timus. Kesimpulan dari kajian ini menunjukkan bahawa sekatan makanan di peringkat awal hayat dapat meningkatkan penghasilan "HSP 70". Pendedahan ayam di peringkat awal hayat kepada tahap sekatan makanan yang terlalu minima (F80) atau terlampau teruk (F40) tidak berjaya menghasilkan ketahanan yang jitu terhadap tegasan haba di peringkat akhir hayat.



## **ACKNOWLEDGEMENTS**

Praises to almighty Allah, the cherisher and sustainer of the world, whose blessings have enabled the author to complete this study.

I am exceedingly grateful to my chairman, Associate Professor Dr. Zulkifli Idrus for his advice, guidance, patience, stimulating discussion, constant encouragement throughout the planning and execution of this study. His sacrifices and perseverance will ever be remembered.

Sincere thanks are also due to Dr. Abdul Rahman Omar and Dr. Daud Israf Ali who have provided advice, helpful discussion and enlightening comments that have improved this study.

I am also thankful to the Director, Institute of Bioscience, Professor Dr. Abdul Rani Bahaman and Associate Professor Dr. Khatijah Mohd Yusoff for granting permission to use equipment and facilities in their laboratory.

I would like to express my deep appreciation and gratitude to the followings that have been of great help in my study: Mr. Ibrahim Mohsin, Mr.

Ponnusamy Muniandy, Mr. Mazlan Hamzah, Mr. O.K. Ho, Mr. Halmi Othman, Iman Rahayu and Mr. Richard Chong Chen Hiung for sharing their technical skills and their ever available assistance. Not forgetting, I would also like to thank all friends and every one who helped me in one way or another.

Finally, I wish to express my deepest appreciation to my father and my mum, sisters Che Faridah, Angah, Amie and CT, brother Him and my fiancé Sobri for their constant support, encouragement, patience and prayers throughout my study in this campus.


I certify that an Examination Committee met on 3 January, 2000 to conduct the final examination of Che Norma Mat Taib on his Master thesis entitled "Heat Shock Protein 70 and Heat Tolerance in Early-Age Feed Restricted Broiler Chickens" in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia Regulations 1981. The committee recommends that the candidate be awarded the relevant degree. Members of the Examination Committee are as follows:

**ZULKIFLI IDRUS, Ph.D.**  
Associate Professor  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Chairman)

**DAUD AHMAD ISRAF ALI, Ph.D.**  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**ABDUL RAHMAN OMAR, Ph.D.**  
Faculty of Veterinary Medicine  
Universiti Putra Malaysia  
(Member)

**ZAINAL AZNAM MOHD. JELAN, Ph.D.**  
Associate Professor/Head  
Department of Animal Science  
Faculty of Agriculture  
Universiti Putra Malaysia  
(Independent Examiner)



**MOHD. GHAZALI MOHAYIDIN, Ph.D.**  
Professor/Deputy Dean of Graduate School  
Universiti Putra Malaysia

Date: 10 MAR 2000

This thesis was submitted to the senate of Universiti Putra Malaysia and was accepted as fulfilment of the requirements for the degree of Master of Science.



**KAMIS AWANG, Ph.D.**

Associate Professor/Dean of Graduate School  
Universiti Putra Malaysia

Date: 11 MAY 2000

## DECLARATION

I hereby declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that this thesis has not been previously or concurrently submitted for any other degree at UPM or any institutions.



**(CHE NORMA BT MAT TAIB)**

Date: 03 . 03 . 2000

## TABLES OF CONTENTS

	PAGE
DEDICATION.....	ii
ABSTRACT.....	iii
ABSTRAK.....	v
ACNOWLEDGEMENTS.....	viii
APPROVAL SHEETS.....	x
DECLARATION FORM.....	xii
LIST OF TABLES.....	xvi
LIST OF FIGURE.....	xvii
LIST OF PLATES.....	xviii
LIST OF ABBREVIATIONS.....	xix
<b>CHAPTER</b>	
<b>I INTRODUCTION.....</b>	<b>1</b>
<b>II LITERATURE REVIEW.....</b>	<b>6</b>
Theory and Concept of Stress.....	6
Heat Stress.....	7
Physiological Responses to Heat Stress.....	8
Heart Rate, Cardiac Output, Blood-Pressure and Total Peripheral Resistance.....	9
Effect of Stress on Performance.....	11
Growth Rate and Feed Efficiency.....	11
Egg Production and Egg Quality.....	12
Blood Glucose, Cholesterol and Mineral Responses to Heat Stress.....	13
Hormonal Response to Stress.....	15
Corticosteroids.....	15
Thyroid Hormones .....	16



Aldosterone.....	17
Reproductive Hormones.....	18
Catecholamines.....	18
Melatonin.....	18
Behavioural Response to Heat Stress.....	19
Feed and Water Intake.....	20
Fear.....	21
Immune Response to Heat Stress.....	23
Programmed Cell Death/Apoptosis.....	24
Effect of Hyperthermia on Lymphocytes.....	26
The Effect of Environment on Heterophil/Lymphocyte (H/L) Response.....	30
Prior Exposure and Response to Subsequent Stressors....	31
Acclimation to High Ambient Temperature.....	32
Heat Shock Proteins.....	35
Introduction.....	35
Effect of Stress on Heat Shock Proteins.....	36
Physiological Functions of Heat Shock Proteins	37
<b>III MATERIALS AND METHODS.....</b>	<b>40</b>
Birds, Husbandry, Experimental Procedure and Traits Measured.....	40
Heat Conditioning and Traits Measured.....	41
Determination of Protein Concentration.....	42
Determination of Molecular Weight of Heat Shock Proteins.....	43
Preparation of the Separating Gel.....	44
Preparation of the Stacking Gel.....	44

	<b>Western Blotting.....</b>	<b>46</b>
	<b>Determination of Apoptotic Cells by Transmission Electron Microscope.....</b>	<b>48</b>
	<b>Blood Glucose Level Detection.....</b>	<b>51</b>
	<b>Blood Cholesterol Level Detection.....</b>	<b>52</b>
	<b>Determination of Heterophil/Lymphocyte Ratios.....</b>	<b>53</b>
	<b>Preparation of Serum Sample for Detection of Antibody Level of Newcastle Disease.....</b>	<b>53</b>
<b>IV</b>	<b>RESULTS.....</b>	<b>56</b>
<b>V</b>	<b>DISCUSSION.....</b>	<b>79</b>
<b>VI</b>	<b>CONCLUDING REMARKS.....</b>	<b>94</b>
	<b>BIBLIOGRAPHY.....</b>	<b>96</b>
	<b>APPENDICES.....</b>	<b>126</b>
	<b>VITA.....</b>	<b>142</b>



## LIST OF TABLES

Table		Page
1	Mean body weights (g) of broiler chickens by feeding regimen at various ages .....	58
2	Mean weekly feed intake (g) by feeding regimen at various ages.....	59
3	Mean weekly feed conversion ratios (feed/gain) by feeding regimen at various ages.....	60
4	Mean serum glucose levels (Mmol/L) by stage of heat treatment and feeding regimen.....	64
5	Mean serum cholesterol level (Mmol/L) where stage of heat treatment by feeding regimen interaction were significant.....	65
6	Mean heat shock protein 70 densities a day following feed restriction.....	66
7	Mean heat shock protein 70 densities where stage of heat treatment by feeding regimen interactions were significant....	68
8	Mean number of apoptotic thymocytes where stage of heat treatment by feeding regimen interactions were significant....	73
9	Mean of antibody titers against Newcastle Disease vaccination where stage of heat treatment by feeding regimen interaction were significant.....	76
10	Mean heterophil/lymphocyte ratios a day following feed restriction.....	77
11	Mean heterophil/lymphocyte ratios where stage heat treatment by feeding regimen interactions were significant....	78

## LIST OF FIGURE

Figure		Page
1	Percentage of survivability of broiler chickens during heat treatment ( $38\pm 1^{\circ}\text{C}$ for 2 hr) from days 35 to 42 .....	61

## LIST OF PLATES

Plate		Page
1	PVDF membrane representation of heat shock protein (HSP 70) synthesis in the brain samples of chicks in response to various levels of feed restriction on Days 4, 5 and 6. Molecular protein marker (MR-kDa) are indicated on the left and HSP 70 on the right.....	67
2	PVDF membrane representation of HSP 70 synthesis in the brain samples of chicks prior to heat treatment by feeding regimen.....	70
3	PVDF membrane representation of HSP 70 synthesis in the brain samples of chicks following 4 days of heat treatment by feeding regimen.....	71
4	PVDF membrane representation of HSP 70 synthesis in the brain samples of chicks following 7 days of heat treatment by feeding regimen.....	72
5	Electron microscope appearance of heat-induced apoptosis in thymus cells. The effected cell shows overall condensation. Note the presence of characteristic nuclear fragments. TEM X 3500.....	74



## LIST OF ABBREVIATIONS

<b>ACTH</b>	Adrenocorticotrophic Hormone
<b>AL</b>	<i>Ad libitum</i>
<b>ATP</b>	Adenosine Triphosphate
<b>BSA</b>	Bovine Serum Albumin
<b>CRF</b>	Corticotrophin-Releasing Factor
<b>CP</b>	Crude Protein
<b>E</b>	Epinephrine
<b>EDTA</b>	Ethylenediaminetetraacetic Acid
<b>ELISA</b>	Enzyme Linked Immunosorbent Assays
<b>FCR</b>	Feed Conversion Ratio
<b>FR</b>	Feed Restriction
<b>hr</b>	Hour
<b>HPA</b>	Hypothalamic-Pituitary-Adrenal
<b>HSP</b>	Heat Shock Protein
<b>HSC</b>	70 kDa Heat Shock Cognate Protein
<b>IgG</b>	Immunoglobulin G
<b>kcal</b>	Kilocalorie
<b>kDa</b>	Kilodalton

<b>kg</b>	Kilogram
<b>LH</b>	Luteinizing Hormone
<b>LHRH</b>	Luteinizing Hormone Releasing Hormone
<b>ME</b>	Metabolisable Energy
<b>min</b>	Minute
<b>mRNA</b>	Messenger Ribonucleic Acid
<b>MT</b>	Mesotocin
<b>NE</b>	Norepinephrine
<b>PBS</b>	Phosphate Buffer Saline
<b>pH</b>	Pulssan Hydrogen (Hydrogen-ion Concentration)
<b>PVDF</b>	Polyvinylidene Difluoride Membrane
<b>PVN</b>	Paraventricular Nucleus of the Hypothalamus
<b>RNA</b>	Ribonucleic Acid
<b>r-T<sub>3</sub></b>	Reverse Triiodothyronine
<b>SDS-PAGE</b>	Sodium Dodecyl -Polyacrylamide Gel
<b>T<sub>3</sub></b>	Triiodothyronine
<b>T<sub>4</sub></b>	Thyroxine
<b>TEM</b>	Transmission Electron Microscope
<b>Tris-HCL</b>	Tris (Hydroxymethyl aminomethane)
<b>V</b>	Volt

$\mu$	<b>Micron</b>
$\mu\text{g}$	<b>Microgram</b>
$\mu\text{l}$	<b>Microliter</b>

## CHAPTER I

### INTRODUCTION

The combined efforts of man and nature have brought tremendous changes in the performance of commercial broiler chickens. Over the period of time, the age to slaughter and the amount of feed required to produce a given quality of meat have been more than halved. However, fast growing broilers are more susceptible to various environmental insults, particularly heat stress. Intense selection for rapid growth rate in commercial meat-type chickens results in concomitant increase in metabolic heat production while heat dissipation capacity is not affected (Sandercock *et al.*, 1995).

For the last several decades, extensive studies have been conducted to combat the dire consequences of thermal stress. These approaches included dietary supplementation of vitamins and electrolytes (Blalock *et al.*, 1984; Kafri and Cherry, 1984; Pardue *et al.*, 1984; Eden and Campbell, 1985; Teerter *et al.*, 1985; Eden, 1986; El-Boushy 1988; Njoku and Nwazota, 1989; Ait-Boulahten *et al.*, 1989; 1993, 1995; McKee *et al.*, 1997), short-term fasting (McCormick *et al.*, 1980a;b; Preston, 1987; Mench, 1992; Zulkifli and Fauzi, 1996), genetic manipulation (Bohren *et al.*, 1981; El-Gendy and Washbun 1989; 1992; Yamada and Tanaka, 1992)

and provision of high fat and low protein diet (Ramlah and Sarinah 1992). Work by Arjona *et al.* (1988; 1990), and Yahav and Hurwitz (1996) demonstrated that exposing five day-old broiler chicks to controlled elevated temperatures enhanced their tolerance to heat stress later in life. An animal does not always have to be preconditioned to the same stressor for habituation to take place. Zulkifli *et al.* (1994a;b) demonstrated that chicks subjected to 60% feed restriction at an early age, were more resistant to marble spleen disease, had better weight gain and lower heterophil to lymphocytes ratios as juveniles than those fed *ad libitum* throughout the experiment in response to high ambient temperatures. The authors suggested that such a husbandry procedure might be more realistic under field situations than prestressing with heat. There is also, the question of whether severity of neonatal stimulation may influence degree of tolerance later in life. To the best of my knowledge, the relationship between severity of early-age stressful experience and tolerance to subsequent insults has not been documented.

Despite the findings that thermal tolerance later in life could be elicited through neonatal fasting, the mechanism involved in the phenomenon is unknown. Zulkifli *et al.* (1994b; 1995) found that neonatal stress without concurrent increases in the synthesis and liberation of glucocorticoid might not help an animal in responding subsequent stressors. However, there is the question of what corticosterone-elicited physiological changes occurred following neonatal stimulation. The



acquired improved thermotolerance by prior exposure to controlled thermal stressors in poultry has been associated with improved heat-shock protein (HSP) response (Eden *et al.*, 1992; Wang, 1992; Wang and Eden, 1993). When prokaryotic and eukaryotic cells are exposed to a variety of physiological stresses such as a non lethal temperature (40–43°C) and heavy metal, the synthesis of most proteins is suppressed but a small number of highly conserved proteins are rapidly synthesized. This reaction is referred to as a "stress response" or "heat shock response" and the induced proteins are called stress proteins or heat shock proteins (HSPs) (Craig, 1985; Lindquist, 1986; Lindquist and Craig, 1988; Morimoto *et al.*, 1990; Craig and Gross, 1991; Nover, 1991). Wang and Eden (1993) suggested that the synthesis rate of the HSP was very low at the control temperature. When the incubation temperature was raised to a level above body temperature ( $T_b$ ), the HSP synthesis rate accelerated dramatically and reached a maximal rate at 45°C for 30 minutes. The response of cells or whole organism to heat shock is extremely rapid but transient, and it involves the redistribution of preformed HSPs within the cell, as well as immediate translation of preformed messenger RNA (mRNA) into HSPs, immediate transcription of genes encoding heat shock proteins and cessation of transcription or translation of other genes or mRNA (Yost *et al.*, 1990). It is widely accepted that one of the most important functions of HSP is to protect organisms from toxic effects of heating (Barbe *et al.*, 1988). In a heat shocked cell, the HSP may bind to heat sensitive proteins and protect them from degradation, or may prevent damaged for